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Indian Standard

SPECIFICATION FOR EDIBLE FULL-FAT SOYA FLOUR

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Indian Standard

SPECIFICATION FOR EDIBLE FULL-FAT SOYA FLOUR

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Indian Standard

SPECIFICATION FOR EDIBLE FULL-FAT SOYA FLOUR

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 30 October 1975, after the draft finalized by the Nutrition Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 During the past few years, an increasing area has gradually been brought under soyabean cultivation, and this is expected to go up further in years to come as crop rotations are further diversified. Soyabean protein is rich in lysine, an essential amino acid and can, therefore, serve as a nutritionally useful ingredient in blended and processed foods. Proteins from soyabean are now being manufactured in the country in many forms, such as high-fat, medium-fat and low-fat flours and protein isolates, and there is scope for developing others. With a view to enable the manufacturers of various types of soya flours in optimizing their operations, as well as to help the users of such flours in the manufacture of processed foods, this standard is being issued.

0.3 Separate Indian Standards are being issued to cover the requirements of full-fat, medium-fat and low-fat edible soya flours and of soya protein isolates. As such the two Indian Standards IS : 5275-1969* and IS : 5276-1969† are being withdrawn.

0.4 To get the best out of soya products which are used as a protein source, they should be heat-treated. Varying the degree of heat treatment of soya flours progressively and simultaneously raises the protein efficiency or quality, inactivates such enzymes as trypsin inhibitors, urease and lipoxygenases, and lowers the nitrogen solubility index. Such heat treatment can be applied to all soya protein products, whether full-fat, medium-fat, low-fat or isolates. Moist heat treatment destroys the several anti-nutritional enzymes present in soyabeans and reduces their activity. Dry heat treatment raises the quality of the protein present, but overheating will lower it. The optimum kind and degree of heat treatment to be applied to soya proteins for use in any particular food product, therefore, depends on the nature and extent of heating which subsequent manufacture of that food product entails, if good protein quality is to be ensured. In order to help food manufacturers, guidelines recommending usage of edible soya flours is given in Appendix A.

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*Specification for edible soya flour (expeller pressed).

†Specification for edible soya flour (solvent extracted).

0.5 This standard has been formulated in close collaboration with the Protein Foods and Nutrition Development Association of India. In the preparation of this standard, due consideration has been given to the provisions of the Prevention of Food Adulteration Act, 1954, and the Rules framed thereunder. However, this standard is subject to the restrictions imposed under these rules, wherever applicable.

0.6 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS : 2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

1. SCOPE

1.1 This standard prescribes the requirements and the methods of sampling and test for edible full-fat soya flour.

2. REQUIREMENTS

2.1 Description — Edible full-fat soya flour shall be obtained from clean, sound, healthy soyabeans by a process of cracking and dehulling, followed by either cooking, drying and grinding or by extrusion, cooking and grinding. Edible full-fat soya flour shall be in the form of coarse or fine powder, or grits; white to creamy white in colour; of uniform composition; and free from rancid odour, extraneous matter and insect, rodent or fungal infestation. The material shall be free from any artificial colouring matter or flavouring agents.

2.1.1 The particle size of the material shall be as agreed to between the purchaser and the vendor.

2.2 The material shall also conform to the requirements given in Table 1.

TABLE 1 REQUIREMENTS FOR EDIBLE FULL-FAT SOYA FLOUR

Sl No.	Characteristic	Requirement	Method of Test, Ref to Appendix of IS: 4684-1975*
(1)	(2)	(3)	(4)
i)	Moisture, percent by mass, *Max*	9·0	B
ii)	Protein (on dry basis), percent by mass, *Min*	39	C
iii)	Total ash (on dry basis), percent by mass, *Min*	6	D
iv)	Acid-insoluble ash (on dry basis), percent by mass, *Max*	0·35	E
v)	Fat (on dry basis), percent by mass, *Min*	16·5	F
vi)	Crude fibre (on dry basis), percent by mass, *Max*	3·5	H
vii)	Aflatoxin, µg/kg, *Max*	30	J

*Specification for edible groundnut flour (expeller pressed) (*first revision*).

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*Rules for rounding off numerical values (*revised*).

2.3 Bacteriological Requirements — The edible full-fat soya flour shall be tested periodically to comply with the requirements given in Table 2.

TABLE 2 BACTERIOLOGICAL REQUIREMENTS FOR EDIBLE FULL-FAT SOYA FLOUR

SL No.	CHARACTERISTIC	REQUIREMENT	METHOD OF TEST, REF TO
(1)	(2)	(3)	(4)
i)	Total bacterial count per g, *Max*	50 000	IS: 5402-1969*
ii)	Coliform bacteria per g, *Max*	10	IS: 5401-1969†
iii)	Salmonella bacteria	Nil	IS: 5887-1970‡

*Method for plate count of bacteria in foodstuffs.

†Methods for detection and estimation of coliform bacteria in foodstuffs.

‡Methods for detection of bacteria responsible for food poisoning and food-borne diseases.

2.4 Premises — Place where edible full-fat soya flour shall be manufactured, packed and stored, and the equipment used shall be maintained under hygienic conditions (*see* IS : 2491-1972*).

3. PACKING AND MARKING

3.1 Packing — Edible full-fat soya flour shall be packed in polyethylene or polyethylene-lined jute bags, or in clean tinplate containers. When packed in bags the mouth of each bag shall be either machine- or hand-stitched. If hand-stitched, the mouth shall be rolled over and stitched. Stitches shall be in two cross-rows with at least 14 stitches in each row.

3.2 Marking — The following particulars shall be marked or labelled on each container:

a) Name of the product,
b) Name and address of the manufacturer,
c) Batch or code number,
d) Net mass,
e) Nitrogen solubility index, and
f) Date of manufacture.

3.2.1 If the flour is a totally unheated product it should be stated on the label.

3.2.2 Each container may also be marked with the ISI Certification Mark.

NOTE — The use of the ISI Certification Mark is governed by the provisions of the Indian Standards Institution (Certification Marks) Act and the Rules and Regulations made thereunder. The ISI Mark on products covered by an Indian Standard conveys

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*Code for hygienic conditions for food processing units (*first revision*).

the assurance that they have been produced to comply with the requirements of that standard under a well-defined system of inspection, testing and quality control which is devised and supervised by ISI and operated by the producer. ISI marked products are also continuously checked by ISI for conformity to that standard as a further safeguard. Details of conditions under which a licence for the use of the ISI Certification Mark may be granted to manufacturers or processors, may be obtained from the Indian Standards Institution.

4. SAMPLING

4.1 Representative samples of the material shall be drawn and tested for conformity to this standard as prescribed in IS: 5315-1969*.

5. TESTS

5.1 Tests shall be carried out as prescribed in col 4 of Tables 1 and 2.

5.2 Quality of Reagents — Unless otherwise specified, pure chemicals and distilled water (*see* IS : 1070-1960†) shall be employed in the tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the test results.

APPENDIX A

(*Clause* 0.4)

GUIDELINES FOR PRODUCT USAGE

A-1. SELECTION OF EDIBLE FULL-FAT SOYA FLOUR

A-1.1 For selecting right quality of the material depending on the end use of the product, guidelines for protein and enzyme quality are given in Table 3.

A-2. TESTS

A-2.1 Determination of the nitrogen solubility index, trypsin inhibitor activity and urease activity shall be carried out according to the method prescribed in Appendices B, C and D.

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*Methods of sampling for milled cereals and pulses products.

†Specification for water, distilled quality (*revised*).

TABLE 3 GUIDELINES FOR SELECTION OF EDIBLE FULL-FAT SOYA FLOUR FOR SPECIFIC END USES

(*Clause* A-1.1)

SL No.	PRODUCTS IN WHICH FLOUR IS USED	RELATIVE PROTEIN EFFICIENCY	NITROGEN SOLUBILITY INDEX	TRYPSIN INHIBITOR ACTIVITY, UNITS	UREASE TEST, DIFFERENCE IN *p*H
(1)	(2)	(3)	(4)	(5)	(6)
i)	Baked goods as a bleaching agent (bread, biscuits)	45	90 *Min*	90 *Min*	Above 1·0
ii)	Products involving moist cooking (bread, biscuits, buns, cakes, extruded foods)	55 to 65	80 to 60	75 *Max*	0·6 to 0·9
iii)	Products involving dry cooking or roasting, and which will not require further cooking or will only be lightly cooked before consumption (protein mixes, powders, protein-fortified cereals and flours, fried snacks)	65 to 75	60 to 40	55 *Max*	0·3 *Max*
iv)	Products involving simple mixing of ingredients with little or no cooking, and which will be consumed without any cooking (protein powders or mixes, baby foods, animal feeds)	80	30 *Max*	40 *Max*	0·05 *Max*
v)	Products in which dispersibility is essential (beverages, youghurt, wipped emulsions or cream, certain pharmaceuticals)	90	90 *Max*	Negligible	Negligible

NOTE — Relative protein efficiency is expressed as percentage of the full potential of soya protein.

APPENDIX B

(*Clause* A-2.1)

DETERMINATION OF NITROGEN SOLUBILITY INDEX

B-0. GENERAL

B-0.1 The method is of special application to the determination of the nitrogen solubility index of soya proteins in the form of ground soyabeans, full-fat, medium-fat and low-fat protein products, and protein isolates.

B-1. APPARATUS

B-1.1 Stirring Apparatus — electrically operated and adjustable up to 120 rpm. The stirrer paddle should have the segment of a circle, and maximum diameter of 50 mm.

B-1.2 Centrifuge — provided with 50 ml round-bottom centrifuge tubes.

B-1.3 Standard Kjeldahl Equipment — as described in Appendix C of IS : 4684-1975*.

B-1.4 Water-Bath — capable of maintaining temperature of 30 ± 0·5°C.

B-2. REAGENTS

B-2.1 Silicone Antifoam

B-2.2 Glass Wool

B-2.3 Standard Kjeldahl Reagents — as described in Appendix C of IS : 4684-1975*.

B-3. SAMPLE PREPARATION

B-3.1 For soyabeans or full-fat soya flour, use a mill which will grind the material to a flour so that at least 80 percent of the material will pass through a 180-micron IS Sieve and 90 percent through a 250-micron IS Sieve (*see* IS : 460-1962†).

B-4. PROCEDURE

B-4.1 Weigh 5 g (accuracy 0·01 g) of the sample into a 400-ml beaker. Measure out 200 ml of distilled water which is at 30°C. Add the water in small portions, dispersing it thoroughly with a glass rod. Stir in the remainder of the water, using the last portion to wash off the rod.

B-4.2 Stir the mixture at 120 rpm using the mechanical stirrer for 120 minutes with the beaker immersed in a water-bath maintained at 30°C. At the end of stirring, transfer the contents to a 250-ml volumetric flask, carefully washing out all the contents of the beaker. Add one or two drops of silicone, make up to the mark with distilled water, and mix the contents of the flask thoroughly by several inversions.

B-4.3 Allow to stand for a few minutes and decant off about 40 ml into a 50-ml centrifuge tube. Centrifuge for 10 minutes at 1 500 rpm. Decant the supernatant liquid carefully (not disturbing the centrifuged solids at the bottom) into a funnel containing a plug of glass wool, and collect the clear filtrate in a 100-ml beaker.

B-4.4 Pipette out 25 ml of the clear liquid into a Kjeldahl flask and determine the quantity of nitrogen present following the procedure described in Appendix C of IS : 4684-1975*. Also determine total nitrogen of the ground sample (*see* **B-3.1**) using the same method described in Appendix C of IS : 4684-1975*.

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*Specification for edible groundnut flour (expeller pressed) (*first revision*).

†Specification for test sieves (*revised*).

B-5. CALCULATION

B-5.1 Water-soluble nitrogen, percent by mass $= \dfrac{(V_1 - V_2) \times N \times 0{\cdot}014 \times 100}{M}$

where

V_1 = volume in ml of alkali for back-titration of blank,
V_2 = volume in ml of alkali for back-titration of sample,
N = normality of alkali, and
M = mass of the sample.

B-5.2 Nitrogen solubility index (NSI) $= \dfrac{\text{Water-soluble nitrogen}}{\text{Total nitrogen}} \times 100$

NOTE — Differences between determinations in the same laboratory should not exceed 3·7 units, and between two laboratories 7·2 units.

APPENDIX C

(*Clause* A-2.1)

DETERMINATION OF TRYPSIN INHIBITOR ACTIVITY

C-0. GENERAL

C-0.1 The method is applicable to the determination of the levels of trypsin inhibitor activity in whole soyabeans and in various soya protein products. Trypsin inhibitor activity is evaluated by measuring the extent to which the ability of trypsin to digest casein is retarded by the inhibitor present in soya products. The substrate (casein) is digested with trypsin in the presence and absence of trypsin inhibitor for a specified period. The enzyme action is stopped and the undigested protein precipitated with trichloroacetic acid (TCA). The extent of proteolysis is determined by measuring the absorption of the TCA filtrate at 280 μ.

C-1. APPARATUS

C-1.1 Homogenizer or Blender or Mechanical Shaker — of suitable type.

C-1.2 Water-Bath — capable of maintaining temperature of 37 ± 0·5°C.

C-1.3 Centrifuge — provided with 20-25 ml round-bottom centrifuge tubes; rpm 3 000.

C-1.4 Spectrophotometer — of suitable type, capable of use in the ultraviolet region.

C-2. REAGENTS

C-2.1 Casein Solution — 2·0 percent. Prepare 0·1 M phosphate buffer solution (*p*H 7·6) by dissolving 23·3 g of disodium hydrogen phosphate ($Na_2HPO_4 . 7H_2O$) and 1·8 g of sodium dihydrogen phosphate ($NaH_2PO_4 . H_2O$) in 900 ml distilled water, adjusting the *p*H to 7·6 with a solution of either a strong acid or a strong base, and making up to 1 litre. Dissolve 2 g of soluble casein in 80 ml of the phosphate buffer by warming on a water-bath for about 15 minutes. Cool the solution and make up to 100 ml with buffer. Store in a refrigerator. The solution is stable for about a week.

C-2.2 Trypsin Solution — Weigh accurately about 4 to 5 mg of a high-grade trypsin (twice-crystallized, salt-free) and dissolve in 100 ml of 0·001 M hydrochloric acid. The solution keeps in the refrigerator for 2 weeks without loss of activity.

C-2.3 Trichloroacetic Acid (TCA) Solution — Dissolve 5 g of trichloroacetic acid in distilled water and make up to 100 ml.

C-3. PROCEDURE

C-3.1 Extracting Trypsin Inhibitor from Soya Products — When necessary, grind the soya product without generating excessive heat (*see* **B-3.1**) so that 60 percent passes through a 425-micron IS Sieve* (equivalent to No. 40 U.S. Standard Sieve). Prepare a one percent extract of the soya products by blending or homogenizing 1 g of the sample with 100 ml of phosphate buffer for about 10 minutes or using a mechanical shaker for about 1 hour. Centrifuge the suspension at 3 000 rpm for 15 minutes. Prepare a 0·2 percent solution by diluting 20 ml of the supernatant to 100 ml with phosphate buffer.

C-3.2 Level of Trypsin to be Used — Pipette 0·2, 0·4, 0·6, 0·8 and 1·0 ml of trypsin solution into a triplicate set of test tubes for each level. Add phosphate buffer to each tube to make up the volume to 2·0 ml. Place the test tubes in a water-bath at 37°C for 5 minutes for temperature equilibration. To one tube in each triplicate set, add 6·0 ml of trichloroacetic acid solution; these tubes serve as blanks. Add 2 ml of casein solution previously brought to 37°C to the first test tube, start the stop-watch, and at 30-second intervals, add 2 ml of casein solution to each of the remaining test tubes, mix well and incubate at 37°C for exactly 20 minutes.

C-3.2.1 The enzyme action is stopped in all the experimental tubes (that is, the two duplicates at each level, excepting the control tube wherein TCA solution had been added initially) by adding 6 ml of 5 percent TCA solution. Remove the test tubes from the water-bath, stopper them and shake vigorously to mix the contents. Set aside for 30 minutes to attain room

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**See* IS : 460-1962 Specification for test sieves (*revised*).

temperature. Centrifuge or filter the contents of the test tubes. Read the optical density of the solutions in a spectrophotometer at 280 nm against the corresponding blank of each. The absorbance reading (A) is raised to $(A)^{3/2}$ and the values are plotted against the concentration of trypsin at each level.

C-3.2.2 From the plot, which should be linear, ascertain the aliquot amount of trypsin solution which yields an optical density of about 0·5. Use this aliquot in determining the trypsin inhibitor activity of the material under test.

C-3.3 Determination of Trypsin Inhibitor Activity — Pipette 0·2 to 1·0 ml aliquots of the extract of the soya product (0·2 percent solution), in steps of 0·2 ml, each in triplicate, into 15 test tubes. Add the chosen level of trypsin solution to all the test tubes. Make up the solution in each test tube to 2·0 ml with phosphate buffer. Place all the tubes in a water-bath at 37°C for 5 minutes for temperature equilibration. To one tube in each triplicate set, add 6·0 ml of trichloroacetic acid solution; these tubes serve as blanks. Add 2 ml of casein solution, previously brought to 37°C, to the first test tube, start the stop-watch and, at 30-second intervals, add 2 ml of casein solution to each of the remaining test tubes.

C-3.3.1 After exactly 20 minutes of incubation at 37°C, to the remaining experimental tubes of each triplicate set, at 30-second intervals, add 6·0 ml of trichloroacetic acid solution. Remove the test tubes from the water-bath, stopper them and shake vigorously to mix the contents. Set aside for 30 minutes to attain room temperature. Centrifuge or filter the contents of the test tubes. Read the optical density of the filtrate in a spectrophotometer at 280 nm against the corresponding blank of each and raise the absorbance values (A) to $(A)^{3/2}$. Calculate the trypsin inhibitor activity per ml of the extract as follows:

Level of Soyabean Extract (ml)	*Absorbance** (A)	$A^{3/2}$	TU†	TUI‡	TUI/ml
0·0	0·542	0·399	40·0	—	—
0·2	0·479	0·331	33·1	6·9	34·5
0·4	0·394	0·247	24·7	15·3	38·2
0·6	0·279	0·148	14·8	25·2	42·0
0·8	0·179	0·076	7·6	32·4	40·5
1·0	0·099	0·031	3·1	36·9	36·9
				Average	38·4

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*Average of duplicate determinations.

†Trypsin unit. One trypsin unit is arbitrarily defined as an increase of 0·01 absorbance units at 280 nm in 20 minutes per 10 ml of the reaction mixture under the conditions set forth in the method.

‡Trypsin inhibitor activity is defined as the number of trypsin units inhibited (TUI).

C-3.4 Dilution Factor in the Case of Processed Soya Flours and Products — In case of processed samples having low trypsin inhibitor activity, the extract should be diluted so that 1 ml would produce 70-80 percent of inhibition of the tryptic activity under the conditions of the assay.

C-4. CALCULATION

C-4.1 Trypsin Inhibitor Activity per g of Sample or per g of Nitrogen in Sample — Trypsin inhibitor activity per gram of the sample (on dry basis) is calculated based on the dilution factor of the extract used in the assay.

C-4.2 Trypsin inhibitor activity per gram of nitrogen in the sample is calculated based on the determination of nitrogen content of the sample (*see* Appendix C of IS : 4684-1975*).

APPENDIX D

(*Clause* A-2.1)

DETERMINATION OF UREASE ACTIVITY

D-0. GENERAL

D-0.1 The method is of special applicability to the urease activity in soyabeans, or protein products derived from them like full-fat, medium-fat and low-fat flours, and isolates. Loss of urease activity at high levels is sensitive to improvement in protein quality, but measurement of urease activity at low levels is a less sensitive index of protein quality.

D-1. APPARATUS

D-1.1 Water-Bath — capable of maintaining temperature of 30 ± 0·5°C.

D-1.2 *p*H Meter — fitted with glass and calomel electrodes and equipped to test 5 ml of solutions.

D-1.3 Test Tubes — 20 × 150 mm, fitted with ground glass or rubber stoppers.

D-1.4 Volumetric Flask — 1 000 ml capacity.

D-2. REAGENTS

D-2.1 Phosphate Buffer Solution — 0·05 M. Dissolve 3·403 g of potassium dihydrogen phosphate ($KH_2PO_4.7H_2O$) in approximately 100 ml of freshly-distilled water. Dissolve 4·355 g of dipotassium hydrogen phosphate ($KH_2PO_4.H_2O$) in approximately 100 ml of water. Combine the two solutions and make up to 1 000 ml. If reagents are pure, the *p*H should be 7·0. If not, before using, adjust the *p*H of the solution with either a solution of strong acid or a strong base. The buffered solution has a useful life of less than 3 months.

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*Specification for edible groundnut flour (expeller pressed) (*first revision*).

D-2.2 Buffered Urea Solution — Dissolve 15 g of urea in 500 ml of phosphate buffer solutions. Add 5 ml of toluene to serve as a preservative and prevent mould formation. Adjust the *p*H of this urea solution to 7·0 using a solution of either a strong acid or a strong base.

D-3. PROCEDURE

D-3.1 Where necessary, grind the sample without generating excessive heat (*see* **B-3.1**) so that 60 percent passes through a 425-micron IS Sieve* (equivalent to No. 40 U.S. Standard Sieve).

D-3.2 Weigh 0·200 g of the material into a test tube. Add 10 ml buffered urea solution, stopper, mix without inverting and place in the water-bath at 30°C.

D-3.3 Place 1 g of the ground sample of the soya product in a small glass beaker or watch-glass and heat it at 135°C for 30 minutes to inactivate the enzyme. Weigh 0·200 g of the inactivated material into a test tube. Add 10 ml of phosphate buffer, stopper, mix without inverting and place in the water-bath at 30°C. It is convenient to place the test and blank test tubes in the water-bath at exactly 5-minute intervals using a stop-watch.

D-3.4 Mix the contents of both the sample and the blank test tubes every 5 minutes during the digestion period. At the end of 30 minutes, remove each tube from the water-bath, transfer the supernatant liquid to a 5-ml beaker and after exactly 5 minutes determine the *p*H.

D-4. CALCULATION

D-4.1 The difference between the *p*H of the test sample (higher) and that of the blank is a measure of the urease activity present in the sample.

NOTE — Fresh raw soyabeans, which give a *p*H change of always over 1 unit, may be usefully run as checks during urease activity determinations.

**See* IS : 460-1962 Specification for test sieves (*revised*).

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AMENDMENT NO. 1 NOVEMBER 1980

TO

IS:7837-1975 SPECIFICATION FOR EDIBLE FULL-FAT SOYA FLOUR

Addenda

(*Page 4, clause 2.1.1*) - Add the following new clause after 2.1.1:

'2.1.2 Edible full-fat soya flour shall be free from harmful foreign oilcakes, such as castor and *MAHUA* when tested according to the methods prescribed in 11 and 12 respectively of IS:7874(Part I)-1975[†]. It shall also be free from *NEEM* cake and other foreign materials such as jaggery and molasses.'

(*Page 4, foot-note with '*' mark*) - Add the following new foot-note after '*' mark:

'[†] Methods of sampling and test for animal feeds and feeding stuffs : Part I General methods.'

[*Page 4, Table 1, Sl No.* (v)] - Add the following new matter under respective columns after Sl No. (v) and renumber the subsequent items accordingly:

'(1)	(2)	(3)	(4)'
vi)	Acid value of extracted fat, *Max*	6.0	4

(AFDC 37)